



(19) Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) Publication number:

**0 312 867 B1**

(12)

## EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **06.05.92** (51) Int. Cl.5: **C07J 9/00, A61K 31/575**

(21) Application number: **88116747.2**

(22) Date of filing: **10.10.88**

---

(54) **Billiary acid derivatives, processes for the preparation thereof and phamaceutical compositions containing them.**

(30) Priority: **20.10.87 IT 2234387**

(43) Date of publication of application:  
**26.04.89 Bulletin 89/17**

(45) Publication of the grant of the patent:  
**06.05.92 Bulletin 92/19**

(84) Designated Contracting States:  
**AT BE CH DE ES FR GB GR IT LI LU NL SE**

(56) References cited:  
**US-A- 4 545 938**

**CHEMICAL ABSTRACTS**, vol. 75, no. 11, 13th September 1971, page 493, abstract no. 77134c, Columbus, Ohio, US; R.A. LEMAHIEU et al.: "Synthesis of some 3,7-dihydroxy-6-methyl-5-pregnene derivatives", & J. MED. CHEM. 1971, 14(7), 629-31

(73) Proprietor: **GIULIANI S.p.A.**  
**Via Palagi, 2**  
**I-20129 Milano(IT)**

(72) Inventor: **Frigerio, Giuliano**  
**Via Palagi, 2**  
**I-20129 Milano(IT)**  
Inventor: **Pellicciari, Roberto Istituto di Chimica Farmac.**  
**Università degli Studi di Perugia Via del Liceo**  
**I-06100 Perugia(IT)**  
Inventor: **Roda, Aldo**  
**Via Alamandini, 9-2**  
**I-40100 Bologna(IT)**

(74) Representative: **Minoja, Fabrizio**  
**Studio Consulenza Brevettuale Via Rossini,**  
**8**  
**I-20122 Milano(IT)**

**EP 0 312 867 B1**

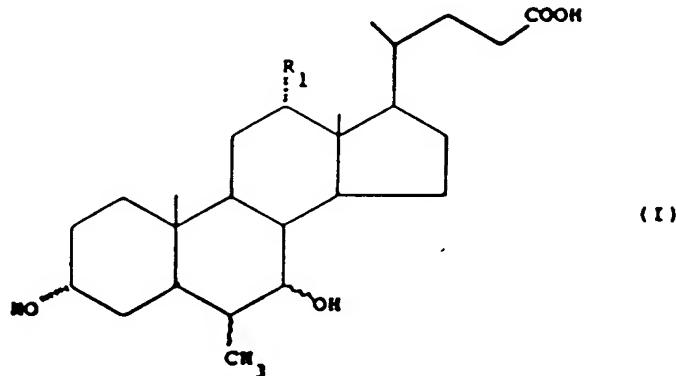
---

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

**Description**

The present invention relates to biliary acid derivatives, to a process for the preparation thereof and to pharmaceutical compositions containing them.

5 The derivatives of the present invention have the following general formula I



wherein R<sub>1</sub> is hydrogen or hydroxy, and the methyl and hydroxy groups at 6- and 7-positions respectively can be either in  $\alpha$  or  $\beta$  configuration.

In other words, compounds I are the 6-methyl derivatives of the following natural biliary acids:

25 ursodeoxycholic (UDCA) 3 $\alpha$ , 7 $\beta$  OH, ursocholic (3 $\alpha$ , 7 $\beta$  OH; R<sub>1</sub> = OH), chenodeoxycholic (3 $\alpha$ , 7 $\alpha$ OH) and cholic (3 $\alpha$ , 7 $\alpha$ OH; R<sub>1</sub> = OH) acids.

The present invention also relates to the physiologically acceptable salts of compounds of formula I, as well as possible glycine or taurine conjugated forms. Moreover, since compounds I can have the methyl group at 6-position as well as the hydroxy group at 7-position either in  $\alpha$  or  $\beta$  configurations, the invention 30 also relates to the single isomers or diastereoisomers and the mixtures thereof.

The above cited biliary acids have been used for a long time in human therapy for the treatment of biliary calculosis, as antidiarrheal, euepeptic, antidyslipidemic and choleric agents, and generally in all those pathological conditions in which a stimulation of biliary flow and a qualitative and/or quantitative change thereof are required.

35 Therapeutic possibilities of natural molecules promoted the development of a number of synthetic or semi-synthetic derivatives in the attempt to obtain improved drugs as regard pharmacokinetic, metabolism or chemico-physical aspects (lipophilicity/hydrophilicity ratio, stability, critical micellar concentration). See, for instance, EP-A- 83106708.7, 84104598.2, 84109811.4, 85115611.7 and USA- P 4648995, 4460509, 4545938.

40 The above cited US patents particularly disclose 7-methyl, 7-hydroxy derivatives which, in comparison with the natural molecules, should provide the advantage of a higher resistance to intestinal bacterial flora, and accordingly a prolonged half-life as well as an increase in stability.

These and other advantages are provided by the compounds of the present invention, which compounds are characterized by the presence of a methyl group at 6-position, 7-position being substantially 45 unchanged in comparison with the natural molecule, which is per se advantageous since 7-position has been found to be critical as regard pharmacological activity.

The methyl group at the 6-position makes the molecule more hydrophobic and more liable to form micelles; this is for example the case of UDCA 6-methyl derivative with respect to UDCA itself.

45 In vitro tests carried out by incubating compounds I with human feces under aerobic conditions, in comparison with UDCA, proved that compounds I have a higher stability and a lower deoxygenation rate than UDCA, and that the half-lives for compounds I and UDCA are respectively of > 24 hours and 8 hours.

Tests effected in rats by intravenous administration of the compounds of the invention at the dose of 2  $\mu$ mol/min/Kg body weight evidenced a choleric effect comparable to that of UDCA and an efficient recovery of the compound in bile. The recovered chemical products are mainly tauro-conjugated forms, and 55 glyco-conjugated forms in a minor part, in a ratio similar to that of UDCA.

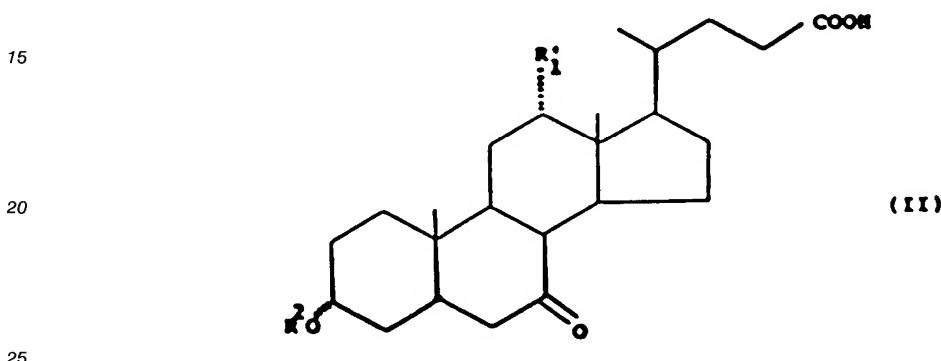
As regards the effect on lipidic biliary secretion, compounds I preferentially decrease cholesterol secretion, keeping constant the phospholipid one.

The compounds of the invention, for the envisaged therapeutical uses, are administered in form of pharmaceutical compositions prepared according to known techniques and excipients, as described e.g. in "Remington's Pharmaceutical Sciences Handbook", Hack Pub. Co., N.Y. USA.

The preferred administration route is the oral one, and the daily doses, which will vary depending on the pathology to be treated and the patient's conditions, will in principle be comprised from 50 to 500 mg, one or more times a day.

Examples of suitable pharmaceutical compositions comprise capsules, tablets, dragees, sugar-coated pills, syrups, granulates, solutions, vials. The compounds of the invention can also be administered by local perfusion, before or after surgical operations, in form of dispersible solutions or powders.

10 The process for the preparation of compounds I consists in the methylation, under controlled conditions, of the compounds of general formula II



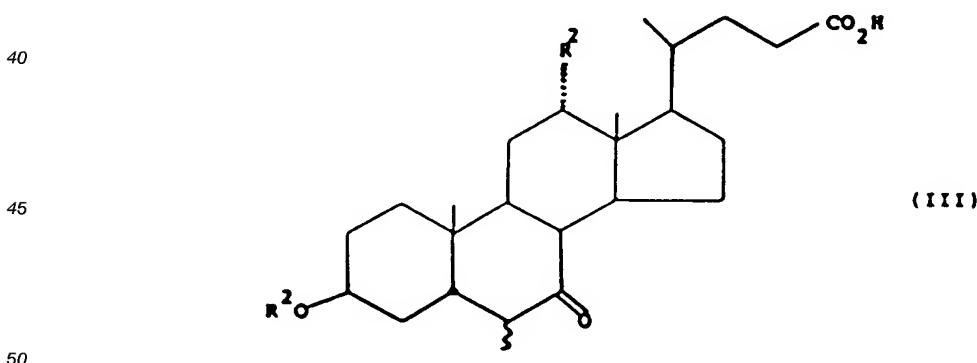
in which R<sub>2</sub> is an hydroxy-protecting group and R'<sub>1</sub> is hydrogen or an hydroxy-protected group.

Methylation is carried out using a methyl halide and appropriate base-solvent systems able to promote the kinetic control in enolate formation.

30 Lithium dialkylamides deriving from secondary amines such as diethylamine, diisopropylamine, piperidine, isopropylcyclohexylamine, hexamethylenedisilazine etc. can be used as the bases in the present invention. Particularly preferred are lithium diisopropylamine or isopropylcyclohexylamine.

Suited solvents are 1,2-dimethoxyethane, tetrahydrofuran, ethyl ether, preferably in the presence of hexamethylphosphoramide (HMPA).

35 The reaction temperature is lower than -50 °C, preferably about -78 °C.  
Resulting compounds III



are then freed from the protecting groups and keto group at 7-position is reduced to 7-hydroxy group.

Any group stable under the reaction conditions can be used as the protecting group. Particularly preferred is the tetrahydropyranyl group. Reduction of keto group can finally be effected by means of conventional reactions, e.g. with metal hydrides or according to Meerwein-Ponndorf.

In case compound III is impure for the presence of unreacted compound II, it is advisable to carry out a chromatographic separation on the methyl ester mixture.

The following non-limiting example illustrates the invention in more detail.

**EXAMPLE****a) 3- $\alpha$ -tetrahydropyranloxy 7-keto-5- $\beta$ -cholan-24-oic acid (II).**

5 P-toluenesulfonic acid (3.00 g, 1.6 mmol) then, slowly, 3,4-dihydro-2H-pyran (DHP) (2.3 g, 27 mmol) were added to a solution of 3- $\alpha$ -hydroxy-7-keto-5- $\beta$ -cholan-24-oic acid (3.00 g, 7.68 mmol) in anhydrous dioxane (55 ml). The reaction mixture was left under magnetic stirring for 15 minutes at room temperature, then it was added with methanol saturated with NH<sub>3</sub> to pH 8-9. The mixture was evaporated under vacuum, the residue was taken up into chloroform and washed with a saturated NaHCO<sub>3</sub> solution (2 x 20 ml). After 10 drying over anhydrous magnesium sulfate and evaporation under vacuum, the residue (3.5 g) was chromatographed on SiO<sub>2</sub> ( $\phi$  4, h 14). By elution with 95:5 CHCl<sub>3</sub>/MeOH polymerization products of dihydropyran were obtained, then by elution with 90:10 CHC<sub>13</sub>/MeOH the desired compound I was obtained (2.7 g), 72% yield.  
 15 H - NMR (CDCl<sub>3</sub>)  $\delta$  : 0.68 (s, C-18 Me, 3H); 0.9 (d, C-21 Me, 3H); 1.17 (s, C-19 Me, 3H); 3.3 - 4.0 (2m, C-2' CH, C-6' CH<sub>2</sub>, 3H); 4.6-4.8 (brs, C-3 CH-OH, 1H).

**b) 3- $\alpha$ -tetrahydropyranloxy-6- $\xi$ -methyl-7-keto-5-B-cholan-24-oic acid (III)**

20 N-butyl lithium (9.25 ml, 1.6 M solution in hexane), then HMPA (2.5 g, 14 mmol) were added to a diisopropylamine solution (1.41 g, 14 mmol) in tetrahydrofuran (THF) (50 ml). The system was cooled to -78 °C and acid II (2.00 g, 4 mmol) in THF (20 ml) was slowly added. 5 Minutes after the end of the addition, methyl iodide (17.1 g, 12 mmol) was added dropwise. The reaction mixture was then left to warm to room temperature, then it was acidified with 10% HCl and extracted with chloroform (3 x 20 ml). The combined organic phases were washed with water, dried over sodium sulfate and evaporated under 25 vacuum. The crude compound (2.00 g) was chromatographed on SiO<sub>2</sub> ( $\phi$  4, 12 hours). By elution with 98:2 CHCl<sub>3</sub>/MeOH, 1.95 g of a mixture of the starting compound II and the methyl derivative III was obtained.

**c) Separation of methyl 3- $\alpha$ -hydroxy-6- $\xi$ -methyl-7-keto-5-B-cholanoate.**

30 The mixture of II and II (g 2.52, mmol 5.12) obtained in b) was dissolved in THF (4 ml), added with some drops of 37% HCl and stirred for 30 minutes at room temperature; then it was poured into water and extracted with chloroform (2 x 25 ml). The combined organic portions were dried over anhydrous sodium sulfate and evaporated under reduced pressure. 2.00 g of a mixture consisting of the starting compound and deprotected compound III were obtained. The mixture was dissolved in methanol (200 ml) and p-toluenesulfonic acid (0.400 g) was added to the resulting solution. After slow magnetic stirring at room 35 temperature for 12 hours, solvent was evaporated off under vacuum, the residue was taken up into chloroform and washed with water (2 x 20 ml). The organic phases were dried over anhydrous sodium sulfate and the crude product was flash chromatographed on SiO<sub>2</sub> ( $\phi$  5, 20 h). By elution with chloroform, 1.00 g (50%) of the title compound was obtained. H-NMR (CDCl<sub>3</sub>)  $\delta$  : 0.68 (s, C-18 Me 3H); 0.9 (d, C-21 Me, 3H); 1.17 (s, C-19 Me, 3H); 3.6 (s, CO<sub>2</sub> CH<sub>3</sub>, 3H) and 0.98 g of VI (49%).  
 40

**d) 3- $\alpha$ -hydroxy-6- $\xi$ -methyl-7-keto-5-B-cholan-24-oic acid.**

45 The ester obtained in c) (0.900 g, 2.1 mmol) was refluxed for 3 hours in a 10% KOH solution in methanol (20 ml). After cooling, the reaction mixture was acidified with 10% HCl and extracted with ethyl acetate (3 x 15 ml). The combined organic phases were washed with water (2 x 10 ml), dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was chromatographed on SiO<sub>2</sub>, eluting with 90:10 CHCl<sub>3</sub>/MeOH, 0.85 g was obtained (98% of the title acid, m.p. 95-98 °C; H-NMR (CDCl<sub>3</sub>)  $\delta$  : 0.68 (s, C-18 Me, 3H); 3.15-3.55 (m, C-3 CH-OH, 1H); 3.8-4.0 (brs, C-3 CH-OH, 1H).  
 50 Mass spectrum (50 and V) m/e 404.2 - 386.7- 292.9230.0 - 216.1 - 117.4 - 83.7.

**e) 3- $\alpha$ -7- $\xi$ -dihydroxy-6- $\xi$ -methyl-5-B-cholan-24-oic acid**

55 The compound obtained in d) (0.460 g, 1.13 mmol) was dissolved in sec-butanol (15 ml); the mixture was refluxed and added with metal sodium (0.460 g). 2 hours after, the reaction mixture was left to cool, diluted with 5 ml of water, acidified with 37% HCl and extracted with ethyl acetate (3 x 10 ml). The combined organic fractions were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified on SiO<sub>2</sub> ( $\phi$  2.5, h 18). By elution with 98:2 CHCl<sub>3</sub>/Me OH, 0.200 g of the

acid I (44%) was obtained.

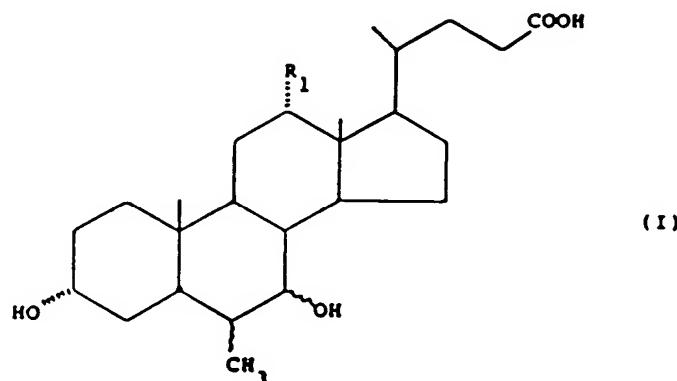
m.p. 128-132 °C. H-NMR (CDCl<sub>3</sub> - CD<sub>3</sub> OD) δ : 0.7 (s, C-18 Me, 3H); 1.00 (t, C-19 and C-6 Me, 6H); 3.45-3.80 (m, C-3 CH-OH, 1H).

5    **Claims**

**Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE**

10    **1. Compounds of general formula I**

15



20

25

in which R<sub>1</sub> is hydrogen or hydroxy, and the methyl and hydroxy groups at the 6- and 7-positions respectively, can be either in α or β configuration.

30

**2. 3-α -7-dihydroxy-6-methyl-5-β-cholan-24-oic acid.**

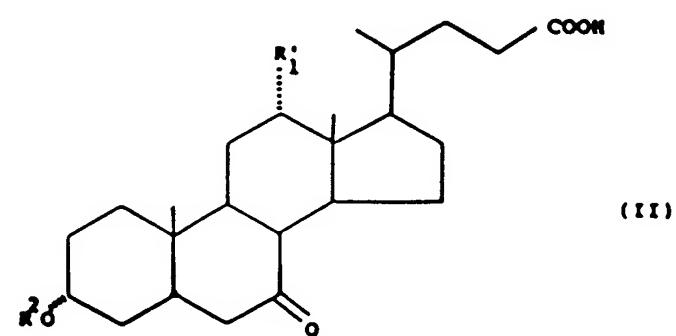
35

**3. 3-α -7-12-α -trihydroxy-6-methyl-5-β-cholan-24-oic acid.**

35

**4. A process for the preparation of the compounds of general formula I, in which process a compound of general formula II**

40



45

50

in which R<sub>2</sub> is a hydroxy-protecting group and R'<sub>1</sub> is hydrogen or a protected hydroxy group, is subjected to methylation under kinetic control conditions, and the resulting compound is subsequently deprotected and reduced.

55

**5. A process according to claim 4, in which methylation is carried out with methyl iodide in the presence of lithium dialkylamides and in solvents selected from the group consisting of 1,2-dimethoxyethane, tetrahydrofuran, ethanol.**

**6. Compounds of formula I according to claims 1-3 as therapeutical agents.**

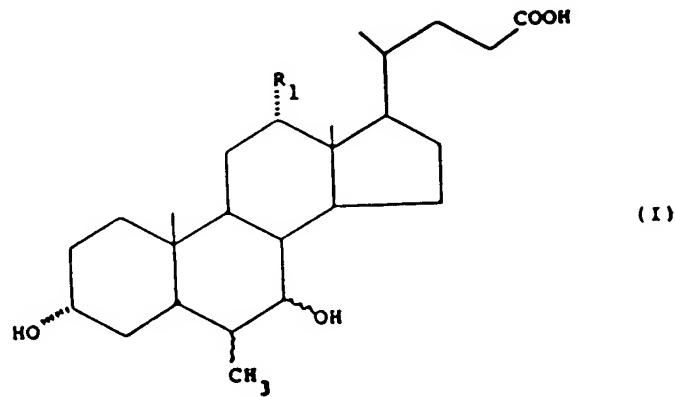
7. Pharmaceutical compositions containing as the active ingredient one compound according to claims 1-3.

8. Use of one compound according to claims 1-3 for the preparation of a medicament having antidiarrhetic, eupeptic, antidyslipidemic and choleretic activities.

**Claims for the following Contracting States : ES, GR**

1. A process for the preparation of compounds of general formula I

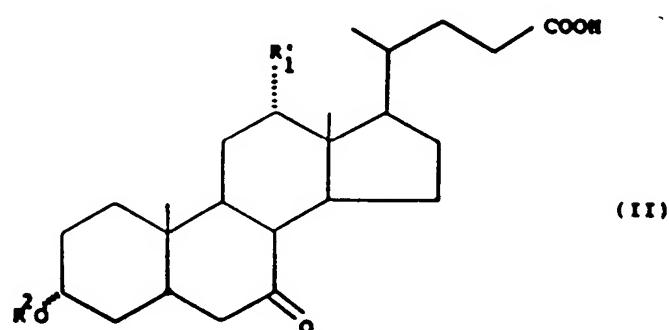
10



in which R<sub>1</sub> is hydrogen or hydroxy, and the methyl and hydroxy groups at the 6- and 7-positions respectively, can be either in  $\alpha$  or  $\beta$  configuration,

in which process a compound of general formula II

30



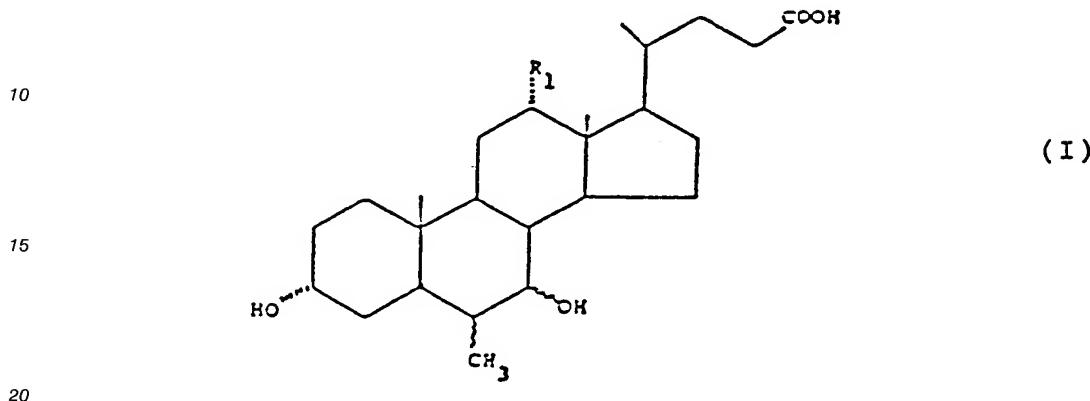
45 in which R<sub>2</sub> is a hydroxy-protecting group and R'<sub>1</sub> is hydrogen or a protected hydroxy group, is subjected to methylation under kinetic control conditions, and the resulting compound is subsequently deprotected and reduced.

50 2. A process according to claim 1, in which methylation is carried out with methyl iodide in the presence of lithium dialkylamides and in solvents selected from the group consisting of 1,2-dimethoxyethane, tetrahydrofuran, ethanol.

55

**Revendications****Revendications pour les Etats contractants suivants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE**

5    1. Composés de formule générale I

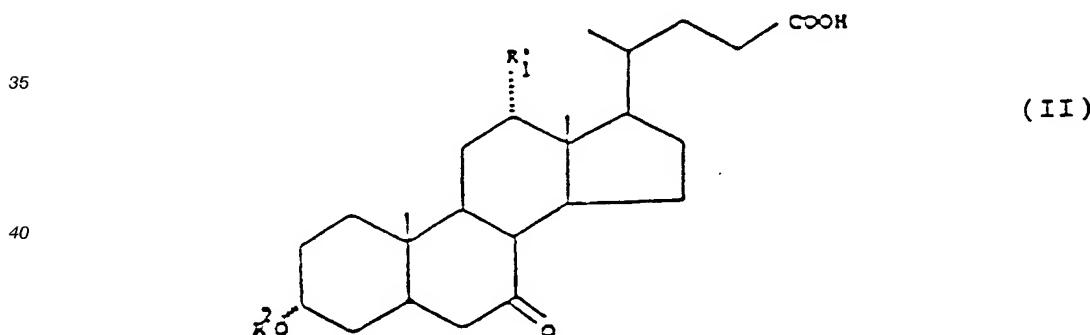


dans laquelle R<sub>1</sub> est l'hydrogène ou hydroxy, et les groupes méthyl et hydroxy en position 6 et 7 respectivement peuvent être soit en configuration  $\alpha$  ou  $\beta$ .

25    2. Acide 3- $\alpha$ -7-dihydroxy-6-méthyl-5- $\beta$ -cholan-24-oïque.

3. Acide 3- $\alpha$ -7-12- $\alpha$ -trihydroxy-6-méthyl-5- $\beta$ -cholan-24-oïque.

4. Un procédé pour la préparation des composés de formule générale I, dans lequel un composé de  
30    formule générale II



dans laquelle R<sub>2</sub> est un groupe protecteur du radical hydroxy et R'<sub>1</sub> est l'hydrogène ou un groupe hydroxy protégé, est soumis à la méthylation sous des conditions de contrôle cinétique et le composé résultant est ensuite déprotégé et réduit.

50    5. Un procédé selon la revendication 4, dans lequel la méthylation est effectuée avec de l'iодure de méthyle en présence de dialcoylamides de lithium et dans des solvants choisis dans le groupe constitué par le 1,2-diméthoxyéthane, le tétrahydrofurane et l'éthanol.

6. Composés de formule I selon les revendications 1 à 3 en tant qu'agents thérapeutiques.

55    7. Compositions pharmaceutiques contenant comme ingrédient actif un composé selon les revendications 1-3.

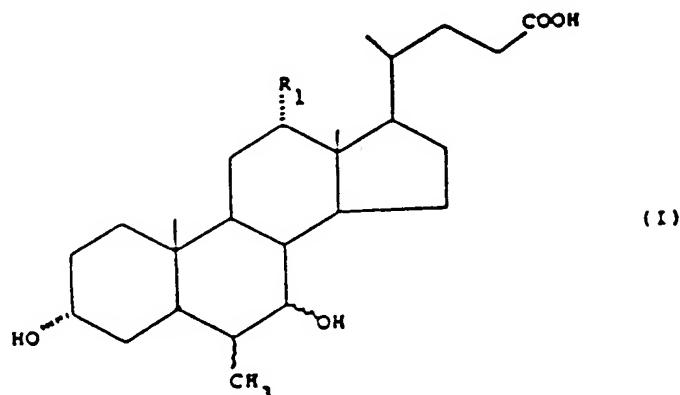
8. Utilisation d'un composé selon les revendications 1-3 pour la préparation d'un médicament ayant des activités antidyspeptiques, eupeptiques, antidiplidémiques et cholérétiques.

**Revendications pour les Etats contractants suivants : ES, GR**

5

1. Un procédé pour la préparation de composés de formule générale I

10



15

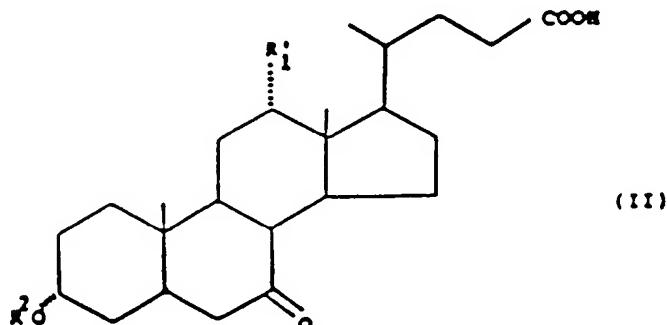
20

dans laquelle R<sub>1</sub> est l'hydrogène ou hydroxy, et les groupes méthyl et hydroxy en position 6 et 7 respectivement peuvent être soit en configuration  $\alpha$  ou  $\beta$ , dans lequel un composé de formule générale II

30

35

40



45

dans laquelle R<sub>2</sub> est un groupe protecteur du radical hydroxy et R'<sub>1</sub> est l'hydrogène ou un groupe hydroxy protégé, est soumis à la méthylation sous des conditions de contrôle cinétique et le composé résultant est ensuite déprotégé et réduit.

50

2. Un procédé selon la revendication 1, dans lequel la méthylation est effectuée avec de l'iode de méthyle en présence de dialcoylamides de lithium et dans les solvants choisis dans le groupe constitué par le 1,2-diméthoxyéthane, le tétrahydrofurane et l'éthanol.

**Patentansprüche**

**Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE**

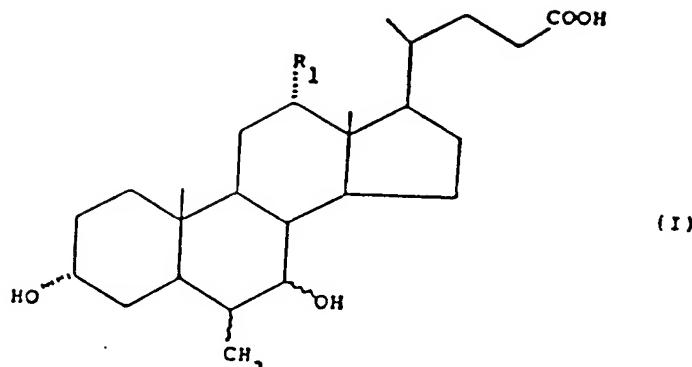
55

## 1. Verbindungen der allgemeinen Formel I

5

10

15



worin R<sub>1</sub> ein Wasserstoffatom oder eine Hydroxylgruppe darstellt, und die Methyl- und Hydroxylgruppen in der 6- bzw. 7-Stellung entweder in der  $\alpha$ - oder  $\beta$ -Konfiguration vorliegen.

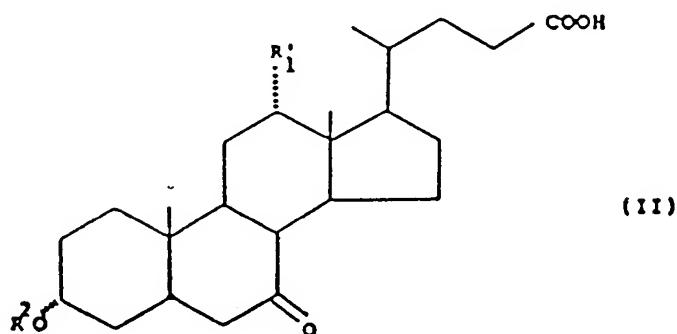
20

2. 3- $\alpha$ -7-Dihydroxy-6-methyl-5- $\beta$ -cholan-24-säure.
3. 3- $\alpha$ -7-12- $\alpha$ -Trihydroxy-6-methyl-5- $\beta$ -cholan-24-säure.
4. Verfahren zur Herstellung der Verbindungen gemäß der allgemeinen Formel I, wobei eine Verbindung der allgemeinen Formel II

30

35

40



in welcher R<sub>2</sub> eine Hydroxyl-Schutzgruppe ist und R<sub>1'</sub> ein Wasserstoffatom oder eine geschützte Hydroxylgruppe darstellt, unter Bedingungen kinetischer Kontrolle einer Methylierung unterworfen wird,

und von der erhaltenen Verbindung nachfolgend die Schutzgruppe abgespalten wird und die Verbin-

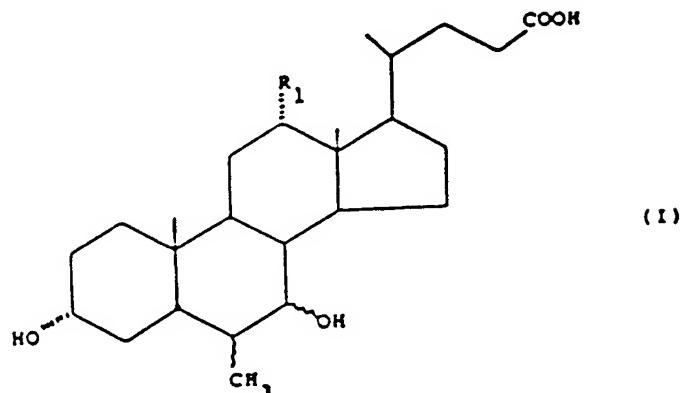
45 dung reduziert wird.

5. Verfahren nach Anspruch 4, worin die Methylierung mit Methyljodid durchgeführt wird in Gegenwart von Lithiumdialkylamiden und in Lösungsmitteln, die ausgewählt sind aus der Gruppe bestehend aus 1,2-Dimethoxyethan, Tetrahydrofuran und Ethanol.
- 50 6. Verbindungen der allgemeinen Formel I gemäß Ansprüchen 1 bis 3 als therapeutische Mittel.
7. Pharmazeutische Zusammensetzungen, welche eine der Verbindungen nach den Ansprüchen 1 bis 3 als aktiven Bestandteil enthalten.
- 55 8. Verwendung einer Verbindung nach den Ansprüchen 1 bis 3 zur Herstellung eines Arzneimittel mit antidyspeptischer, eupetischer, antidyslipidämie-und choleretischer Wirksamkeit.

## Patentansprüche für folgende Vertragsstaaten : ES, GR

## 1. Verfahren zur Herstellung von Verbindungen der allgemeinen Formel I

5



10

15

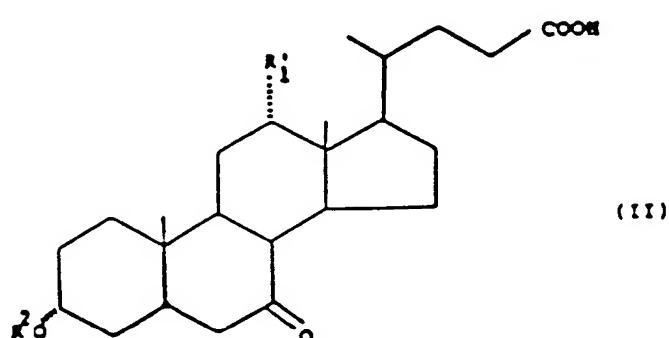
20

vorin R<sub>1</sub> ein Wasserstoffatom oder eine Hydroxylgruppe darstellt, und die Methyl- und Hydroxylgruppen in der 6- bzw. 7-Stellung entweder in der  $\alpha$ - oder  $\beta$ -Konfiguration vorliegen, wobei eine Verbindung der allgemeinen Formel II

25

30

35



40

45

in welcher R<sub>2</sub> eine Hydroxyl-Schutzgruppe ist und R'<sub>1</sub> ein Wasserstoffatom oder eine geschützte Hydroxylgruppe darstellt, unter Bedingungen kinetischer Kontrolle einer Methylierung unterworfen wird, und von der erhaltenen Verbindung nachfolgend die Schutzgruppe abgespalten wird und die Verbindung reduziert wird.

50

55

2. Verfahren nach Anspruch 1, worin die Methylierung mit Methyljodid durchgeführt wird in Gegenwart von Lithiumdialkylamiden und in Lösungsmitteln, die ausgewählt sind aus der Gruppe bestehend aus 1,2-Dimethoxyethan, Tetrahydrofuran und Ethanol.